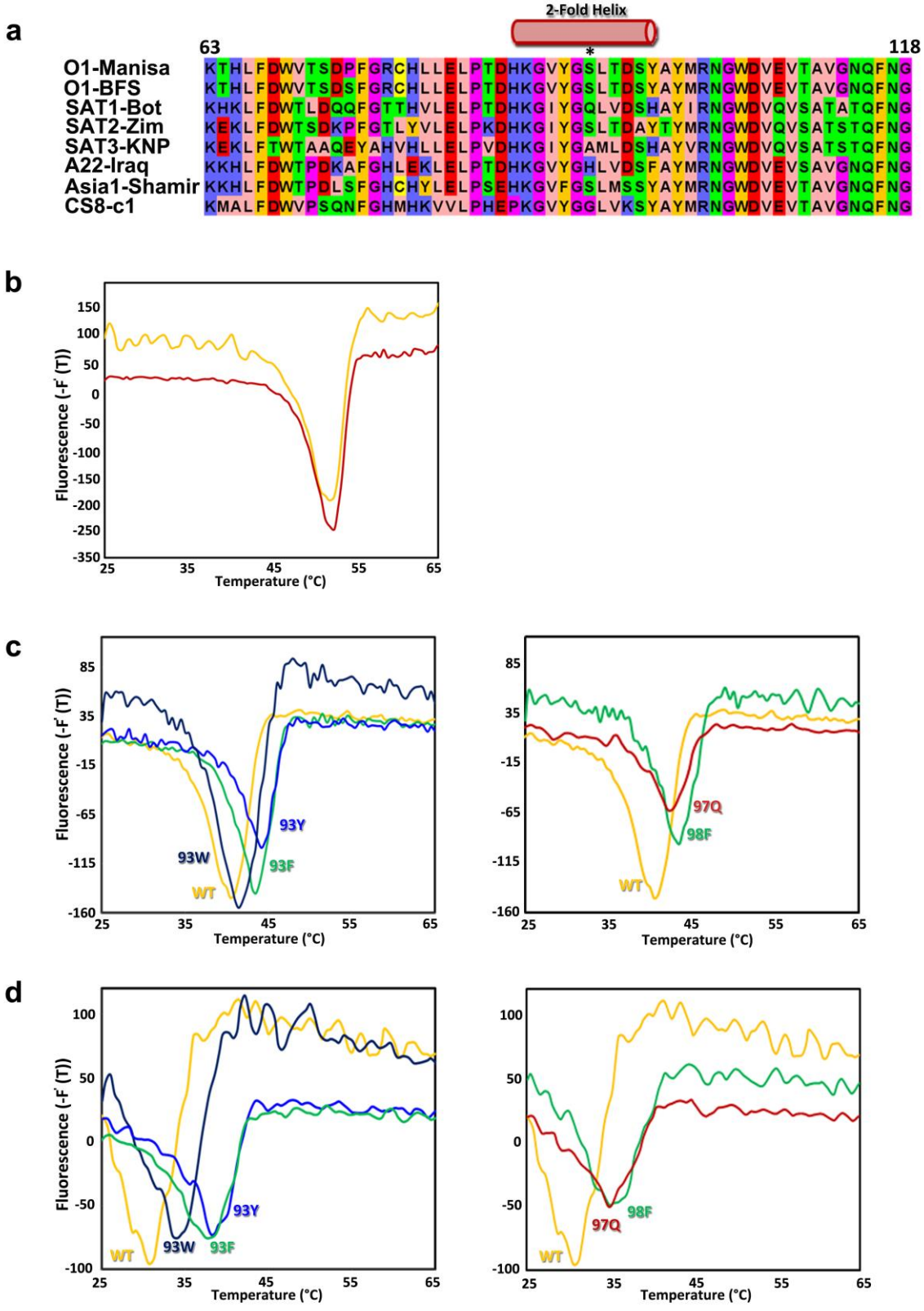
Supplementary Figure 1

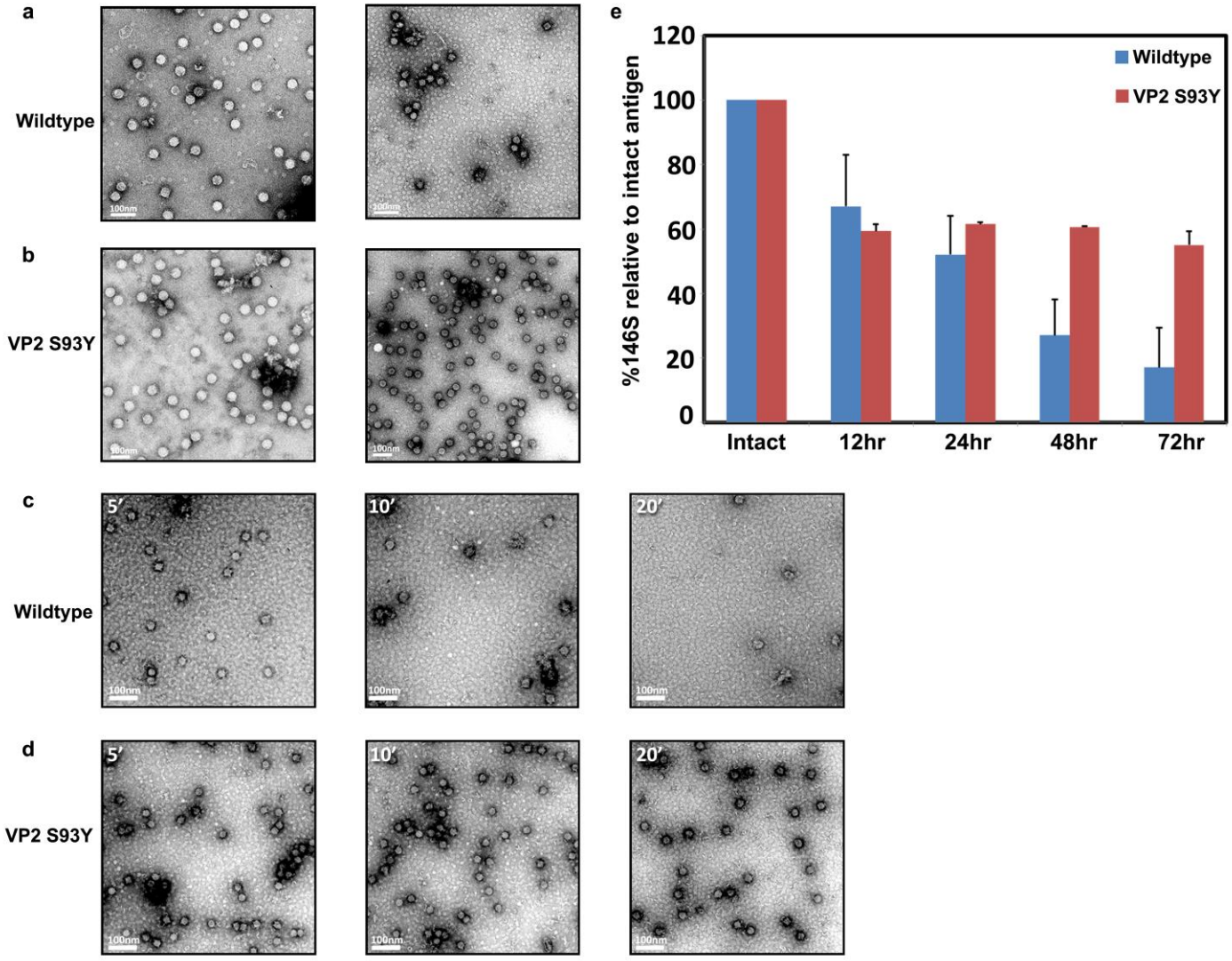


Supplementary Figure 1

Sequences and thermostability of cell culture adapted particles at different pH values.

(**a**) Sequence alignment of a region of VP2 from representatives of seven FMDV serotypes (VP2 dominates the interactions at the inter-pentamer interface). The position of the α-helix found in all picornaviruses adjacent to the icosahedral 2-fold symmetry axis (residues 87-98) is shown as a red cylinder with position 93 indicated by a star (amino acid sequences are coloured using the Zappo Colour scheme in Jalview, http://www.jalview.org/). (**b**) The thermostability of infectious O1M wild type viruses with (HS+) and without (HS-) cell adaptation mutations remote from the pentamer interface were measured by fluorescence assay at pH 7.5. Both HS+ and HS- dissociated at 52.5˚C. (**c**) The engineered mutants are noticeably more thermostable at lower pH. At pH 7.0, the wild-type capsids dissociated at 41.0˚C (compared to 52.0˚C at pH 7.5, Figure 2b), S93Y dissociated at 43.5˚C, S93F at 42.5˚C, S93W at 41.5˚C, S97Q at 42.5˚C and Y98F at 43.4˚C. (**d**) At pH 6.5, the wild-type capsids dissociated at 30.5˚C, S93W dissociated at 34.0˚C and S93Y and S93F at 38.0˚C, whilst S97Q and Y98F dissociated at 34.0˚C.

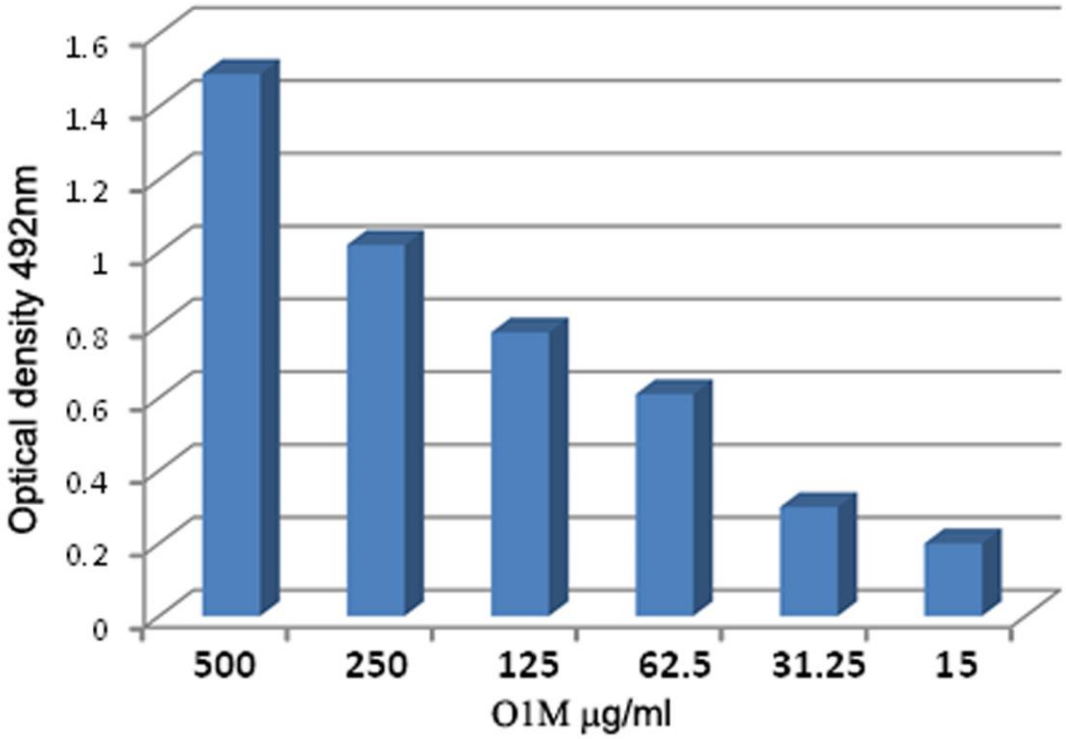
Supplementary Figure 2



Supplementary Figure 2

Effect of storage and heat treatment on inactivated wild-type and engineered O1M virus.

Purified inactivated O1M wild-type and S93Y mutant were analysed for intactness by negative stain EM. (**a**) ~10% of wild-type capsids are dissociated into pentamers when analysed soon after purification (quantified by taking the mean count of 5 independent areas on the grid), whereas S93Y particles appeared mostly intact. (**b**) Aliquots of the purified particles were stored at 4˚C and analysed again after ten days. ~80% of the wild-type capsids are dissociated into pentamers, whilst only ~10% dissociation is observed for the S93Y mutant (**c**) When incubated at 37˚C, wild-type O1M capsids readily dissociated into pentamers. After 20 min only ~5% capsids remained intact (taking the mean count of 5 independent areas on the grid compared to a similar mean count at time 0). (**d**) In contrast, S93Y mutant capsids were more resistant to heat treatment and ~60% capsids remained intact after 20 min. The scale bar indicates 100nm. (**e**) To further test the effect of storage, infectious O1M wild-type and mutant S93Y capsids were incubated at 37˚C for up to 72 h and aliquots analysed at 12, 24, 48 and 72 h post incubation by ELISA using single domain llama antibodies that specifically detect intact capsids (146S) or dissociated pentamers. Results are expressed as % of 146S particles relative to the intact antigen (starting antigen). After 72 h, ~50% mutant capsids remained intact whereas for wild-type ~15% capsids remained whole.

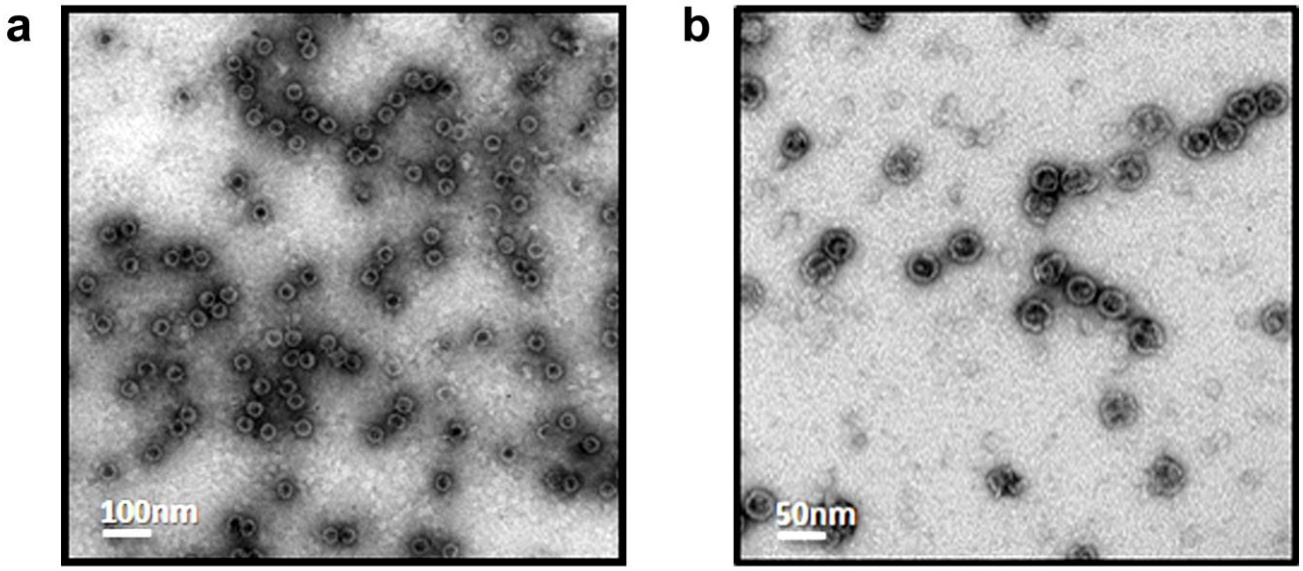


Supplementary Figure 3

Indirect sandwich ELISA for detection of FMDV O1Manisa.

The guinea-pig polyclonal antisera to FMDV O1M used in this study was generated by immunization with inactivated, purified 146S (FMD) virus particles in Freund's Complete as shown previously (Ferris and Donaldson, 1984) and used in an indirect sandwich ELISA for FMDV antigen detection within the FAO World Reference Laboratory for Foot and Mouth Disease (WRL for FMD). Shown is an ELISA (Ferris et al., 2005) using a rabbit anti-FMDV type-O polyclonal serum as a trapping reagent for detection of a dilution series of inactivated, purified FMDV O1Manisa using the guinea-pig polyclonal antisera to O1Manisa as the primary antibody. Similar results were obtained for guinea-pig polyclonal antisera against FMDV A22.

Supplementary Figure 4

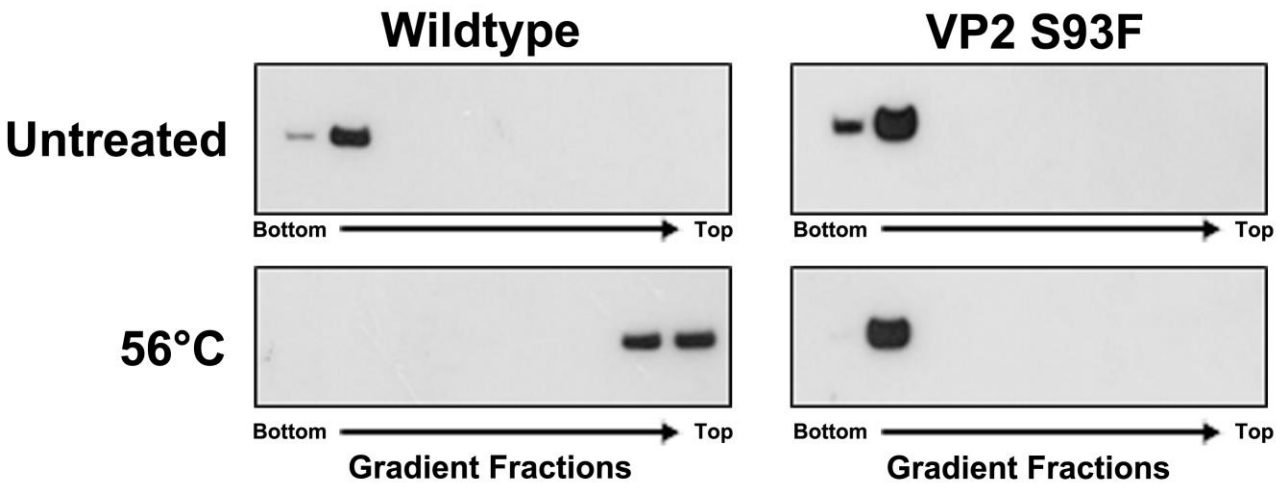


Supplementary Figure 4

Thermostability of engineered O1M recombinant empty capsids.

Recombinant S93F empty capsids were heated to 56˚C for 2 h and sedimented over a 15-45% sucrose gradient. The peak fraction was analysed by EM. (**a**) The empty capsids were found to be intact, further confirming their improved stability. The scale bar indicates 100nm. (**b**) High magnification image showing that ~90% capsids are intact. The scale bar indicates 50nm.

Supplementary Figure 5

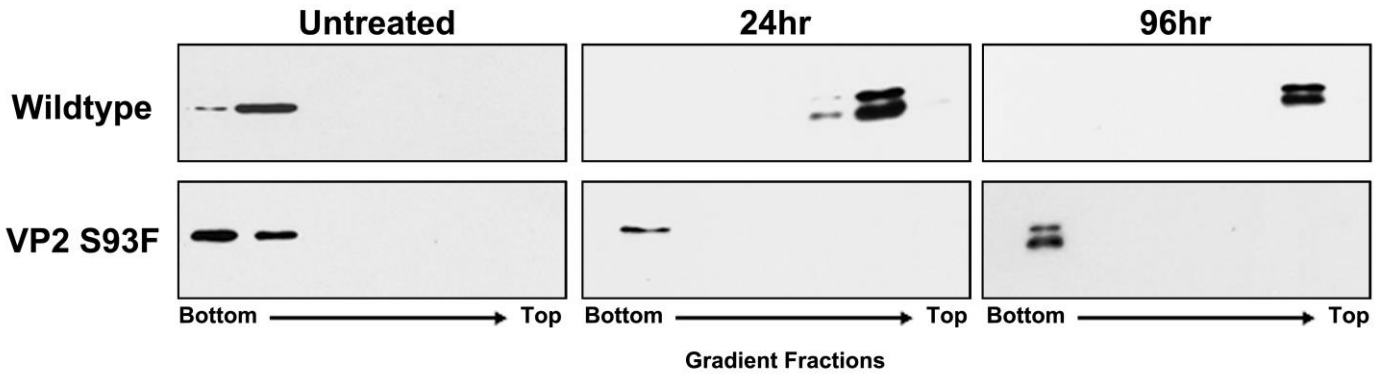


Supplementary Figure 5

Thermostability of engineered A22 recombinant empty capsids.

Purified capsids were left untreated or heated to 56˚C for 2 h and sedimented on 15-45% sucrose density gradients. Fractions were taken from the bottom of the gradients and analysed by western blot. Capsid proteins were detected using an anti-FMDV A22 polyclonal antibody. Heated H93F capsids remained intact and migrated to fractions 3-4, as did untreated capsids, whereas wild-type capsids dissociated upon heating, remaining near the top of the gradient in fractions 10 and 11.

Supplementary Figure 6

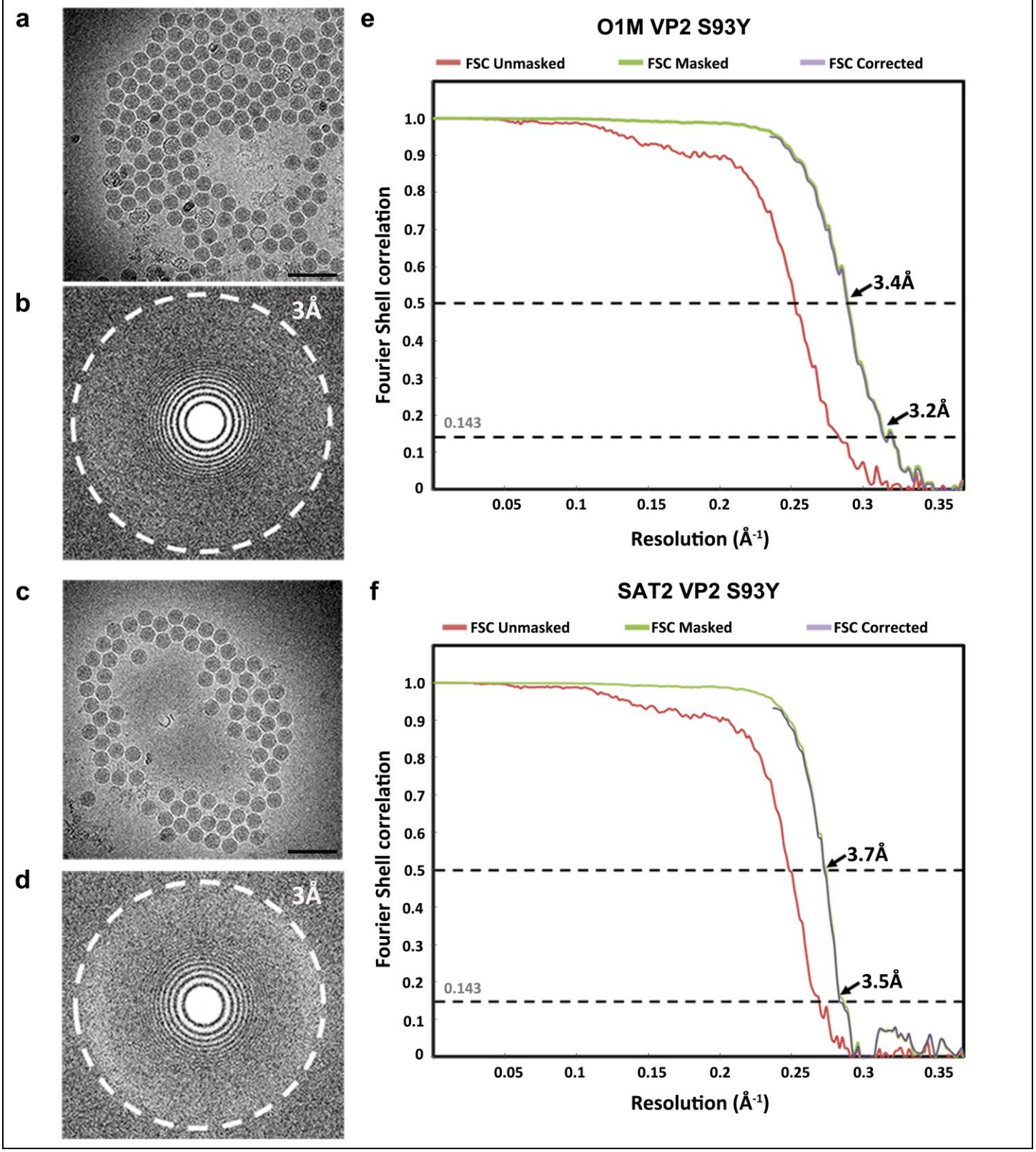


Supplementary Figure 6

Stability of engineered O1M recombinant empty capsids upon storage at 37˚C.

O1M wild-type and S93F empty capsids were incubated at 37˚C for 24 h or 96 h followed by sedimentation on 15-45% sucrose density gradients. Fractions were taken from the bottom of the gradients and analysed by western blot. Capsid proteins were detected using an anti-FMDV O1M polyclonal antibody. Wild-type capsids readily dissociated and remained near the top of the gradient in fractions 10-11, whereas mutant S93F capsids remained intact, even after 96 h, and were detected in fraction 4.

Supplementary Figure 7



Supplementary Figure 7

CryoEM analysis of stabilised FMDV particles.

Purified inactivated O1M and SAT2 S93Y particles were used for data collection by cryoEM. (**a**) and (**b**) Representative aligned average (motion corrected) image of O1M VP2 S93Y particles and the corresponding Fourier transform. (**c**) and (**d**) Representative aligned average (motion corrected) image of SAT2 VP2 S93Y particles and the corresponding Fourier transform. (**e**) and (**f**) FSC curves of the final 3D reconstruction obtained using gold-standard refinement using RELION, marked with the resolution corresponding to a Fourier shell correlation (FSC) of 0.5 and 0.143. The scale bar indicates 100nm.